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Viral Immune Evasion in Dengue: Toward Evidence-Based Revisions of Clinical Practice Guidelines

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Abstract:
Dengue, a leading cause of illness and death in the tropics and subtropics since the 1950's, is fast spreading in the Western hemisphere. Over 30% of the world's population is at risk for the mosquitoes that transmit any one of four related Dengue viruses (DENV). Infection induces lifetime protection to a particular serotype, but successive exposure to a different DENV increases the likelihood of severe form of dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS). Prompt supportive treatment lowers the risk of developing the severe spectrum of Dengue-associated physiopathology. Vaccines are not available, and the most effective protective measure is to prevent mosquito bites. Here, we discuss selected aspects of the syndemic nature of Dengue, including its potential for pathologies of the central nervous system (CNS). We examine the fundamental mechanisms of cell-mediated and humoral immunity to viral infection in general, and the specific implications of these processes in the regulatory control of DENV infection, including DENV evasion from immune surveillance. In line with the emerging model of translational science in health care, which integrates translational research (*viz.*, going from the patient to the bench and back to the patient) and translational effectiveness (*viz.*, integrating and utilizing the best available evidence in clinical settings), we examine novel and timely evidence-based revisions of clinical practice guidelines critical in optimizing the management of DENV infection and Dengue pathologies. We examine the role of tele-medicine and stakeholder engagement in the contemporary model of patient-centered, effectiveness-focused and evidence-based health care.

Abbreviations: BBB: blood-brain barrier; CNS: central nervous system; DAMP: damage-associated molecular patterns; DENV: dengue virus; DF: dengue fever; DHF dengue hemorrhagic fever; DSS dengue shock syndrome; DALYs: isability adjusted life years; IFN-γ: interferon-gamma; ILX: interleukinX; JAK/STAT: janus kinase (JAK) / Signal transducer and activator of transcription (STAT); LT: Escherichia coli heat-labile enterotoxin formulations deficient in GM1 binding by mutation (LT[G33D]); MCP-1: monocyte chemotactic protein 1; M-CSF: macrophage colony-stimulating fact; MHC: major histocompatibility complex; MIF: macrophage migration inhibitory factor; MIP-1]-α / -β: macrophage inflammatory protein-1 alpha and beta; mAb: monoclonal antibody; NS1: non-structural protein 1 of dengue virus; NK: natural killer cells; PAMP:pathogen-associated molecular patterns; PBMC: peripheral blood mononuclear cells; TBF-β: transforming growth factor-beta; TNF-α: tumor necrosis-alpha; VHFs: virus hemorrhagic fevers; WHO: World Health Organization

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Keywords: Dengue virus, Dengue fever, Dengue shock syndrome, immune evasion, M1 & M2 macrophages, Tregs, TH17, translational science, syndemics, tele-medicine, stakeholders engagement, patient-centered research, patient-centered evaluation, disability adjusted life years, comparative effectiveness analysis

Background:

Dengue

Dengue Virus Transmission

Dengue is a systemic viral infection, with remarkable rapid emergence and global spread in the last decades. Dengue virus (DENV) is transmitted to humans by the *Aedes* mosquitoes (Genus: *Aedes*; Subgenus: *Stegomyia*), primarily *Ae. aegypti* and *Ae. albopictus*, as vectors for domestic and peri-domestic transmission, and arboreal Aedes mosquitoes as vectors for enzootic transmission. Tropics and subtropics distribution of these two major vectors puts nearly a third of the global human population at risk of DENV infection **[1].** Dengue is brought about by infection with one of the four genetically related but antigenically distinct serotypes of dengue virus (DENV): DENV 1, DENV 2, DENV 3 and DENV 4, and possibly a fifth dengue virus, which however does not share the same pattern of transmission cycle in humans. DENV, a single-stranded, positive-sense, RNA arbovirus of *Flavivirus genus* (family *Flaviviridae*) with a genome of approximately 11 Kb, established endemic transmission among tropical human populations in the last several hundred years, becoming a worldwide problem since the 1950s **[2].** The transmitting mosquito must feed on an infected person during a 5-day period of high viremia, during which the person is in the process of becoming symptomatic. Asymptomatic individuals can still infect mosquitoes. The mosquito incubates the virus for another additional 8-12 days before being able to transmit it to another human. The mosquito remains infected for the remainder of its lifespan, and can repeatedly transmit DENV. There is scant evidence of transmission in organ transplants, blood transfusions, or from an infected pregnant mother to her fetus.

Dengue was first documented in the Americas at the end of the eighteenth century, and its arrival on this continent, from the forests of Central and West Africa, may have resulted from the slave trade. Although *Aedes* mosquitoes are common in the southern U.S. and Puerto Rico, nearly all Dengue cases reported in the 49 continental states are brought in the US by travelers. These imported cases can lead to significant outbreaks, such as Puerto Rico (1915, 2007), Hawaii (2001) and Texas (2005). Intensive mosquito control measures deployed in the 1960s and 1970s could not prevent, or contain Dengue penetration in the Gulf of Mexico (Cuba, 1981), and the following large epidemic. The infection is endemic in over 100 countries in Asia, the Pacific Rim, the Americas, Africa, and the Caribbean. World Health Organization (WHO) estimates 50-100 million infections worldwide yearly, including 500,000 cases of dengue hemorrhagic fever (DHF) and 22,000 deaths yearly, mostly among children. Recent public health modeling maps the global distribution of Dengue risk, taking into account recent climate changes, including rainfall, temperature change and urbanization, and draws projections of over 400 million Dengue infections globally per year. This prevalence estimate is alarmingly more than three times the dengue burden estimate of the WHO based on traditional modeling projections **[3].**

Dengue Virus Pathology

The clinical presentations of DENV infections are very broad, ranging in severity from asymptomatic or mild undifferentiated dengue fever (DF) to severe DHF or dengue shocking syndrome (DSS), which can be lethal. Dengue must be considered in the differential diagnoses of illness for all patients who have fever and a history of travel to tropical and subtropical areas within 2 weeks before the onset of symptoms. DF usually lasts 2–7 days and manifests as sudden onset of fever, generalized body ache, myalgia, arthralgia and intense headache. Patients may have sore throat, infected pharynx, conjunctival injection, pain behind the eyes, as well as gastrointestinal symptoms, including vomiting and diarrhea. By contrast, DHF, a rare but more severe form of dengue infection, presents minor or major bleeding phenomena, thrombocytopenia (<100,000 platelets/μL), and evidence of increased vascular permeability (e.g., hemoconcentration [hematocrit increased by >20% from baseline], pleural or abdominal effusions, or hypoproteinemia). The first well-documented outbreak of DHF was described in Manila in 1953-54. DSS results from a critical loss in plasma volume, leading to catastrophic circulatory failure, including narrow pulse pressure (<20 mm Hg), hypotension, and shock. DSS has a fatality rate of approximately 10%.

The principal risk factors for developing DHF include (a) the strain of infecting virus, (b) prior infection with a heterologous serotype, and (c) the patient's age, gender, nutrition and genetic make-up. Following the acute febrile phase, temperature drops and an increase in capillary permeability occur. Clinically significant plasma leakage usually lasts 24–48 hours, with associated leukopenia and thrombocytopenia that can precede this event. Patients without increased capillary permeability tend to improve, but cohorts with increased capillary permeability suffer loss in plasma volume and tend to worsen.

Dengue Disease Prognosis

Early diagnostic markers for both DHF and DSS are lacking. The physiologic mechanisms that aid containment of the infection, recovery and convalescence from DENV remain to be characterized. A vaccine is lacking, infection with one DENV serotype does not trigger an endogenous vaccine: in fact, sequential infections with multiple DENV serotypes put people at greater risk for DHF and DSS **[2].**

Management of severe Dengue depends on symptomatic treatment of hemorrhagic complications and hypovolemic shock, and ought to include acetaminophen, for management of pain and fever. But, acetylsalicylic acid (i.e., aspirin) and other non-steroidal anti-inflammatory agents are contra-indicated because of their anticoagulant properties, which can

precipitate DHF and DSS. All patients with Dengue should be monitored for hypotension and related signs of DHF, because prompt fluid therapy can help reduce morbidity and mortality.

The prognosis of DENV infection is determined by a balance between the rate of viral replication and the efficiency of the immune system viral surveillance for viremia clearance **[4].** Using convalescent gene expression levels as baseline, two distinct groups of host immunity genes emerge **[5].** These include an "early" group of genes associated with innate immunity (i.e., acute pro-inflammatory, activation-inducing), including interferon-gamma (IFN-Ỳ), cytokine-mediated signaling, chemotaxis, and complement activity, which together peaks at day 0-1 following DENV infection, and declines 3-4 days thereafter. In addition, a "late" group of genes associated with cell cycle, emerge about day 4 and peaking by day 5-6 (i.e., proliferation-inducing). The up-regulation of these early innate immune response genes coincides, as it should from an immune surveillance perspective, with a drop in DENV viral replication during day 0-3 of DF. Indeed, gene signatures of DHF can be identified as early as day 1, and document a partial slowing of immune surveillance (i.e., reduced expression of genes associated with antigen processing and presentation, MHC class II receptor, natural killer (NK) and T cell activities, compared to that of DF patients). Taken together, the characteristically broad and dynamic host responses in DENV infected subjects appear to consist of two distinct phases of immune surveillance, with unique transcriptional signatures and footprints and strong potential for early molecular diagnostics **[5].** Since DENV is transmitted by *Aedes* mosquitoes, it follows that the most effective prevention measure for Dengue is avoiding exposure to the mosquito vectors by using repellents, wearing protective clothing, and remaining in well-screened or air-conditioned areas. Preventing exposure to *Aedes* and to DENV infection benefits both indigenous habitants and travelers, and protects society from the emergence of autochthonous Dengue transmission in areas where a competent vector is abundant but DENV is absent.

Dengue Neurocognitive Disease

Infection with DENV leads to debilitating headaches, high fever, a variety of neurological disorders, and seizures, which indicate of the virus involvement in pathology of the central nervous system (CNS). The adverse effects of pro-inflammatory cytokines on CNS function in DENV+ patients may result from (a) persistent inflammation of the CNS through the release of cytokines and chemokines and recruitment of infected monocytes, (b) disruption of the blood brain barrier (BBB), which increases its permeability and deregulation of tight junction proteins, and (c) acquired neurological insults resulting from DENV virus infection. Virus infections, including DENV, engage initial activation of monocytes/macrophages, which release pro-inflammatory cytokines that target endothelial cells, among others, and disrupt the BBB vascular system. This early innate immunity cytokine response also leads to deregulation of homeostatic mechanisms, destruction of host tissues and apoptosis **[6].** Infection of DENV leads to increased synthesis of tumor necrosis-alpha (TNF-α), in large part responsible for the cachexia and the physiopathology observed in patients with virus hemorrhagic fevers (VHFs) in general and Dengue in particular. Infection also leads to the release of a variety of interleukins (e.g., IL1-β, IL6, IL8, IL15, IL16), chemokines (e.g., macrophage inflammatory protein [MIP-1]-α and -β, monocyte chemotactic protein 1 [MCP1]), myeloid growth and migratory factors (e.g., macrophage colony-stimulating factor [M-CSF], macrophage migration inhibitory factor [MIF]), and other myeloid function regulatory factors.

In the brain, receptors for IL1β, IL6, and TNF-α have been most widely studied in relation to neuropsychiatric and neurological disorders. These pro-inflammatory cytokines have atherogenic and prothrombotic effects that directly influence ischemic stroke, vascular dementia and other CNS pathologies. Data show that IL6 alters adult neurogenesis in many neuropathological conditions, including possibly in stroke, *status epilepticus*, Alzheimer, Parkinson and Huntington diseases, putatively by interfering with oxidative stress and apoptosis. IL6 and its downstream JAK/STAT signaling pathway appear to modulate facilitate cognitive flexibility **[7].** By contrast, TNF-α, produced by CNS astrocytes and microglia, alters synaptic transmission and plasticity in several neurological disorders by there inhibitory effect on glutamate transporters, resulting in increased glutamate concentration in the brain, which can affect cognitive processes and behaviors such as sleep, and water and food intake **[8].** MCP-1, a β-chemokine expressed during inflammation, activates its receptor, CCR2, to induce chemotaxis of monocytes to the inflammatory sites. It is a potent activator of macrophages, and its levels are elevated in cerebral inflammation **[9].** Inflammation resulting from viral infection, such as DENV, and mediated in part by chemokine activity and the release of proinflammatory cytokines, contributes to BBB breakdown, and leads to increased risk of viral CNS invasion. CNS perivascular cells also play a key role in brain inflammation by recruiting peripheral blood mononuclear cells (PBMC) to the brain, causing neuronal damage and microglial inflammation.

Evasion of Immune System by Dengue

Innate Immunity

Pro-inflammatory cytokines are also toxic to peripheral tissues and organs, when in chronically elevated and unregulated concentrations. The gastrointestinal and other mucosal linings are targeted first, leading to the observed capillary leakage, renal failure, diarrhea, and disseminated intravascular coagulation: signs of virulent and often lethal hemorrhagic fever. Systemically, DENV infection leads to a rapid initial rise in pro-inflammatory cytokines (e.g., IL6, TNF-α), which trigger a relatively short-lived initial burst of fever and inflammation often disregarded by the patient, and cellular immune migration factors (e.g., IL-8) to recruit PBMC. Soon into the immune surveillance response, which commences immediately after infection, a slower process of cellular pathology ensues, which includes myeloid cell and endothelial cell infection and cytotoxicity. Consequential to this second phase are both a sharp rise in fever, and loss in vascular integrity, which leads to increased permeability of blood vessels with transudates increasingly rich in micronutrients, red blood cells and eventually white blood cells. Deficiencies in specific and nonspecific immune-driven antiviral responses result in unrestricted DENV replication and dissemination in the host, which together lead unavoidably to death within 14 days following the appearance of EVD symptoms. Unless, prompt

intervention are engaged to prevent DENV binding to target cells, or block its replication within infected macrophages and dendritic cells to counter the physiological collapse due to dehydration secondary to heavy bleeding and violent diarrhea.

In brief, DENV finds multiple ways to evade immune surveillance, and actively subverts both innate and adaptive immune responses, in part by triggering harmful inflammatory responses that inflict direct tissue damage **[4, 10].** The organism is ultimately overwhelmed by a combination of inflammatory factors and virus-induced cell damage, particularly in the vasculature, often leading to death from liver and kidney failure, complicated by septic shock. In part for this reason, live-attenuated tetravalent chimeric yellow fever-dengue vaccines generally afford little or no protection against disease caused by DENV-2 in both human and sub-human primates. Live-attenuated tetravalent DENV vaccines also exhibit evidence of immunological interference. It is possible and even probable that vaccine specifically directed to DENV non-structural protein 1 (NS1) may be successful in preventing the more severe forms of the disease **[11].** Macrophages are myeloid derivatives that mature from monocytes and process foreign materials by phagocytosis, a process that has evolved in vertebrate immunology to recognize pathogens and damaged tissues through pattern recognition receptors (PRR)/Toll receptors. They recognize pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP) **[12].**

It is now clear that there are two primary states of mature macrophage activation and function. Macrophages either elicit responses that include nitric oxide and oxygen radical production, the M1 stage, or they may be involved in the production of factors that promote proliferation, angiogenesis, and matrix deposition, referred to as the M2 stage. M1 macrophages actively metabolize arginine either to nitric oxide and citrulline via the inducible nitric oxide synthase pathway: M1 physio-toxic profile; M2 macrophages process arginine to ornithine and urea via the arginase pathway: M2 physio-repairing profile. An M1 stage produces IL12 and IL23, which signal T cells to elicit a TH1 response with high levels of IFN-γ. Thus M1 is sustained, and TH17 activation ensues further amplifying M1-mediated cytotoxicity. Upon containment of the pathogen, M1 macrophages are replaced with M2 that engender a pattern of cytokine associated with TH2 pattern of cytokines to favor tissue remodeling. This includes IL10 that shuts down the TH1 cytokine response, as well as IL4 and IL13 that promote antibody production for the removal of the pathogen, and transforming growth factor-beta (TGF-β), which induces arginase for cell and tissue rebuilding and repair. To a INF-γ/TGB-β-1 and a TH1/TH2 balance seem to correspond a M1/M2 balance, a tissue destruction (by virtue of excessive nitric oxide and related cytotoxic compounds) and a tissue regeneration modality (resulting from arginase-mediated production of polyamines for DNA repair and L-proline and ornithine).. Undoubtedly, the M1/M2 dichotomy is oversimplified description of complex immune-regulatory processes **[13].** However, it is a useful functional classification that simply proposes two different, actually opposing and balancing activities of mature macrophages following viral trigger, such as DENV. If the hypothesis can be brought forward that DENV alters the M1/M2 balance, then, the inference follows that DENV may contribute to drive and sustain the M1 stage simply out of the physiological need to generate new myeloid derivatives to clear DAMP and PAMP. If this hypothetical model were proven true experimentally *in vitro*, it would open new avenues for potential treatment intervention testable *in vivo*.

Case in point, dendritic cells are important antigen presenting cells, and permit such investigations. Studies have shown that targeting certain protein antigens to engage the maturation of dendritic cells can be an efficient means of immunization. Antigen targeting is most often accomplished in these instances by the use of a monoclonal antibody (mAb) directed against a dendritic cell surface receptor fused to the protein of interest.

When this technique is used experimentally to raise immunity against DENV, either of two mAbs (i.e., αDEC205, αDCIR2) are used, which target two distinct dendritic cell subpopulations, distinguished by either DEC205 or DCIR2 endocytic receptors. These mAbs are fused to NS1, and the test animals (e.g., BALB/c mice) are challenged with these conjugated antigens in the presence of the polyriboinosinic:polyribocytidylic acid (poly [I:C]) adjuvant. A strong anti-DENV NS1 immunoglobulin response ensues within a few weeks, indicating that the overall strength of the antibody response does not vary with the different dendritic cell endocytic receptor challenged, whereas remarkable differences in the IgG1/IgG2a ratios can be obtained depending on which of the DEC205 or DCIR2 sites are challenged. Furthermore, the αDEC-NS1 challenge is generally more productive in terms of immune-protective immunoglobulin, rise in the number of IFN-γ producing cells, and involvement of CD4+ and CD8+ immune surveillance cell population, compared to αDCIR2-NS1 targeted dendritic cells **[11].**

T Cell-Mediated Immunity

Potential of anti-DENV DNA vaccines are tested by measuring the protective efficacy and immune responses of mice intramuscularly injected with plasmid encoding DENV NS1. Intravenously challenged by lethal DENV, mice vaccinated with NS1-DNA present a remarkable delay onset of dengue-associated paralysis, a marked decrease of morbidity, and a greater survival. This improved clinical profile is correlated with an elevation of anti-NS1 antibody serum titer, and a strong priming effect on anti-NS1 response, which consists of a vigorous production of naive T cells (CD4+/CD8+CD45RA+), and a strong NS1-specific cytotoxic T cell proliferation and NS1-directed cytotoxicity. Taken together, these concerted immune responses appear to be directed specifically against the non-structural protein of DENV, and are further augmented by co-injection of plasmid encoding the regulatory TH1 cytokine, IL12. This observation unequivocally confirms and establishes the important role for TH1-mediated immune regulation in the effector processes leading to the establishment of immune surveillance to DENV **[14].** It is important to note that T cell-mediated immune surveillance against DENV, as against other viruses, is stringently modulated by the micro-environment, which determines and dictates the intricate and fluid relationships among the different subpopulations of T CD3+ cells, and the pattern of

cytokines they produce. The TH1 and the TH2 patterns of cytokines cited above are regulated by the regulatory T cell subpopulation (Tregs, CD4+/CD8+CD25+FoxP3+). They also respectively engender the TH17 and the TH9 sub-populations, which together modulate and regulate a state of sustained T cell-driven inflammation **[15].** Should the hypothesis that DENV impairs the host's cellular immunity by altering the Tregs-mediated regulation of TH1, TH2, TH17 and TH9 plasticity be proven true, then novel immunotherapies could be designed and tested on DENV+ patients directed specifically at restoring the physiological homeostasis in TH1, TH2, TH17 and TH9 cytokines.

Humoral Immunity

Infection with wild-type DENV induces high-titered neutralizing antibody that can provide long-term immunity to the homotypic virus and short-term immunity (only several months duration) to a heterotypic DENV. The high level of virus replication seen during both secondary infection with a heterotypic virus and during primary DENV infection in late infancy is a direct consequence of antibody-dependent enhancement of replication. This enhanced virus replication is mediated primarily by preexisting, non- or sub-neutralizing antibodies to the virion surface antigens that enhance access of the virion-antibody complex to FcγR-bearing cells. A single amino acid change in DENV envelope protein (e.g., single T51K substitution in the domain I/II hinge region of the viral envelope protein **[16]**; single mutation in domain III of the envelope protein T329A **[17]** confers resistance to a potent antibody through abolishing the antibody-virus interaction. Taken together, these observations are at the basis of an intense program of anti-DENV vaccines development, fueled by the timely and critical need to provide long-term protection against each of the four DENV serotypes by inducing neutralizing antibodies, and live, attenuated and various nonliving virus vaccines **[18].**

For more than a century, immunologists and vaccinologists have existed in parallel universes. Immunologists have for long reveled in using 'model antigens', such as chicken egg ovalbumin or nitrophenyl haptens, to study immune responses in model organisms such as mice. Such studies have yielded many seminal insights about the mechanisms of immune regulation, but their relevance to humans has been questioned. In another universe, vaccinologists have relied on human clinical trials to assess vaccine efficacy, but have done little to take advantage of such trials for studying the nature of immune responses to vaccination. The human model provides a nexus between these two universes, and recent studies have begun to use this patient-centered model to study the molecular profile of innate and adaptive responses to vaccination. Patient systems vaccinology studies provide mechanistic insights about innate and adaptive immunity in humans, including yellow fever and seasonal influenza vaccines.

Converging lines suggest that induction of anti-NS1 immunity correlates with protective immunity, and may generate cross-reactive antibodies that recognize platelets and proteins involved in the coagulation cascade. Bacterial exotoxins, such as *Escherichia coli* heat-labile enterotoxin (LT), exert strong immunostimulaton effects through binding to monosialoganglioside (GM1) cell surface receptors. The LT formulations, deficient in GM1 binding by mutation (LT [G33D]), is a premier promising candidate adjuvant for human trials of parenteral vaccines in general and for current vaccine development. Purified recombinant NS1, jointly administered with the nontoxic Escolar heat-labile enterotoxin LT derivative, may procure the most promising line of new and protein-based anti-dengue vaccines **[19].**

DENV vaccine development includes the use of live, vectored and killed, as well as recombinant preparations. Vaccine candidates must provide broad and robust immunity to all four DENV serotypes simultaneously as secondary DENV infections often enhance disease severity. The design, implementation, and surveillance measures associated with Dengue vaccine trials must be rigorous due to the complexity of the disease and its epidemiology. Eligible trial sites must satisfy several criteria including documented hyper-endemicity and a known epidemiological history of the circulating serotypes. The epidemiological findings from Ratchaburi province in Thailand provide an interesting model in this domain: the data strongly support this location's suitability for a proof-of-concept efficacy trial of the Sanofi-Pasteur tetravalent dengue vaccine **[20].** Accurate disease surveillance and carefully monitored clinical trials will provide essential evidence concerning the efficacy of candidate dengue vaccines, which will hopefully herald a new era in dengue disease prevention **[21].** Case in point, the Sanofi-Aventis© Group, which offers the broadest range of vaccines in the world protecting against 20 bacterial and viral diseases, developed a tetravalent Dengue vaccine, composed of four recombinant, live, attenuated vaccines to the pre-membrane and envelope genes of one of the four DENV serotypes, in a model reminiscent of the yellow fever vaccine. Phase 0 *in vitro*, and Phase 1 *in vivo* preclinical studies have established that the vaccine induces controlled stimulation of human dendritic cells, and significant immune responses in monkeys. Scale up Phase 3 and Phase 4 trials before industrialization in children and adults are yielding conclusive, albeit preliminary results about the vaccine's efficacy and effectiveness. The recommended three-dose vaccination regimen induces an immune response against all four serotypes in the large majority of vaccinees. Preexisting immunity against flavivirus favors a quicker and higher immune responses to the Sanofi-Pasteur tetravalent dengue vaccine, without significant side-effects such as increasing toxicity, excessive viremia, or endangering clinical safety in general. Taken together, these promising outcomes should lead to industrial production and dissemination of the vaccine, and facilitate supply and access to vaccine in the countries where the dengue disease burden makes it an urgent public health priority **[22].**

Oral mucosal vaccination, a feasible and economic vaccination strategy alternative to sub-cutaneous or intramuscular injections, can be an effective method to overcome the pitfalls of current injection-based vaccines, such as pain, high cost of vaccination, risk of infection or cross-contamination. It is a cost-effective vaccine application Ideal for developing countries, which efficiently delivers antigen into mucosal lymphoid organs to trigger a vigorous immune stimulation. But, our knowledge-base presently is prohibitively scares for developing and testing an effective oral anti-DENV vaccine.

Nonetheless, one promising approach for oral mucosal vaccine development is exploring the potential of M cells via M-cell-targeting ligands that have the potential to deliver ligand-conjugated antigens into mucosal lymphoid organs and evoke conjugated-antigen-specific systemic and mucosal immune responses. The M-cell-targeting ligand, Co1, has been tested for inducing specific immune responses against a pathogenic viral antigen, envelope domain III (EDIII) of dengue virus, to provide the foundation for oral mucosal vaccine development against the pathogen. After oral administration of Co1-conjugated EDIII, antigen appears to be effectively delivered to the Peyer's patches. Resulting antibodies induced by the ligand-conjugated EDIII antigen show effective virus-neutralizing activity. Taken together, these observations confirm that the M-cell-targeting strategy using Co1 ligand as a mucosal adjuvant may be a beneficial tool in the pursuit of effective vaccines for pathogenic DENV antigen **[23].**

In brief, antiviral vaccines have been the most successful biomedical intervention for preventing epidemic viral disease, such as Dengue. Recent technological advances in gene delivery and expression, nanoparticles, protein manufacturing, and adjuvants have created the potential for new vaccine platforms that may provide solutions for vaccines against viral pathogens for which no interventions currently exist. The technological convergence of human monoclonal antibody isolation, structural biology, and high-throughput sequencing also provides new opportunities for atomic-level immunogen design. Selection of human monoclonal antibodies can identify immune-dominant antigenic sites associated with neutralization and provide reagents for stabilizing and solving the structure of viral surface proteins. Understanding the structural basis for neutralization can guide selection of vaccine targets. Deep sequencing of the antibody repertoire and defining the ontogeny of the desired antibody responses can reveal the junctional recombination and somatic mutation requirements for B-cell recognition and affinity maturation. Collectively, this information can provide new strategic approaches for selecting DENV vaccine antigens, formulations, and regimens, which together will benefit the development of dengue vaccine programs, and improve our readiness to address this and related new emerging viral threats.

Patient-Centered Outcomes Evaluation and Translational Effectiveness

Recent developments in health care have witnessed the evolution of the original conceptualization of translational research into translational science in medicine, dentistry and nursing. Translational research, as originally defined by NIH, requires that sample biopsies obtained from individual patients be analyzed and characterized in the laboratory, and that the outcome of these studies be integrated in the clinical decision-making for treatment. Translational effectiveness, as later defined by AHRQ, defends that another major component of clinical decision-making must rest on obtaining, disseminating and utilizing the best available evidence in specific clinical settings. Dissemination must be directed in various forms and formats to all stakeholders involved in the patient's well-being - from the patients themselves, to the caregivers, the health-care team, and the patients' friends and acquaintances. Stakeholders play a critical role in the dissemination process, be it person-to-person or via tele-medicine processes, in caring for patients with DENV infection, and the extent on their active engagement in the translational process of health care.

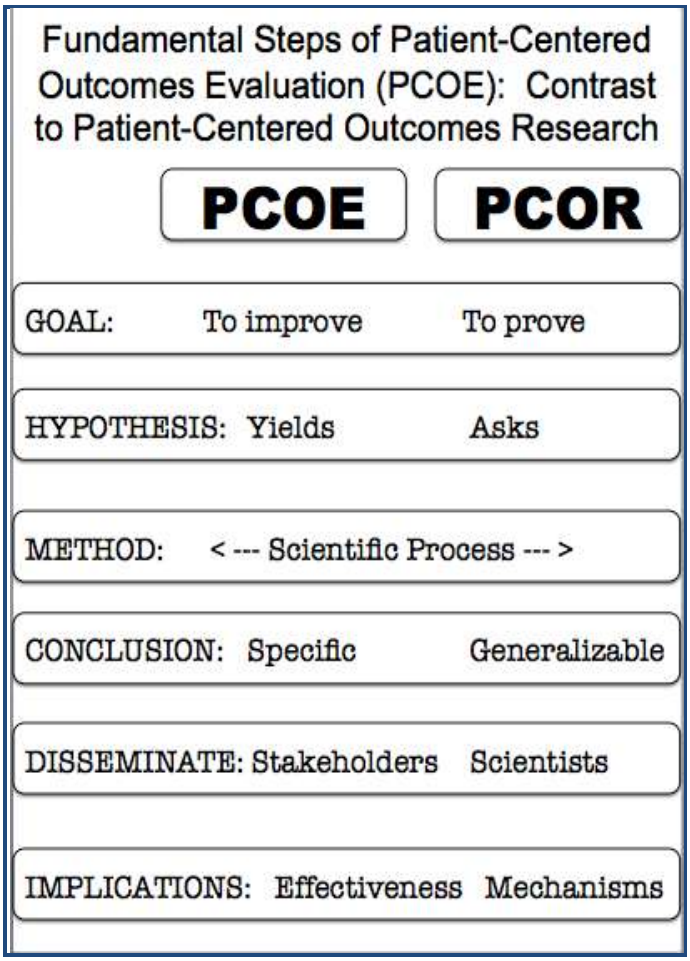

Figure 1: Schematic Representation of the Similarities and Differences between Patient- Centered Outcomes Research and Patient-Centered Outcomes Evaluation

The figure represents a simplified generalization of the fundamental steps of Patient-Centered Outcomes Evaluation (PCOE), in contrast to Patient-Centered Outcomes Research (PCOR). The figure is derived from the ample discussion on this subject provided in the referenced footnote. In brief, PCOE is distinct from PCOR in that the former pursues the goal of improving existing programs, whereas the latter seeks to prove the superiority of one over other programs. In this process, therefore, PCOE generates new hypotheses, whereas PCOR is structured to test existing hypotheses. Whereas both PCOE and PCOR employ the scientific process to reach the conclusions of their respective endeavors, the former obtains conclusions that are specific to the programs under evaluation, but the latter generates conclusions, which, provided the study has strong external validity, will be generalizable beyond the sample under test to the population. Researchers principally disseminate their research outcomes to their peers and fellow researchers in a constant strive to obtain a better, more precise and more accurate understanding of fundamental mechanisms and principles. By contrast, evaluators seek to disseminate their findings to the various stakeholders who are affected, either directly or indirectly by the program under evaluation, with the primary concern of increasing effectiveness – be it

cost-effectiveness of the program under evaluation, or its benefit-effectiveness. From this viewpoint, research and evaluation are two complementary aspect the science of health care, whose interdependence is all the more timely and critical in the context of the contemporary new model of translational science in health care, in which translational research and translational effectiveness are inextricably intertwined. In this light, PCOE and PCOR are the fundamental and indispensable pillars of patient-centered, effectiveness-focussed and evidence-based health care

Despite the rapid advancement in information and communications technology over the last decade, there is limited evidence suggesting improvements in the ability of health professionals to communicate effectively. Given the critical nature of communication, it is timely and critical to initiate further evaluation of information and communication technology designed to improve communication between clinicians **[24].** We recently proposed a framework for systematic patient-centered outcomes evaluation (PCOE) that consisted of six distinct steps, which can be summarized as (a) Focused literature review, (b) Development of draft framework, (c) Workshop with technical experts, (d) Refinement of framework, (e) Development of two case studies, and (f) Pilot test of framework on case studies . The resulting model (**Figure 1**) has several important features combining work from a variety of fields that represent an important step forward in the rigorous assessment of such evidence because it integrates a definition of evidence based on inferential effect, not study design. The model strives to separate evidence about the biological and physiological mechanisms from evidence derived from research synthesis aimed at linking the intervention to a given clinical outcome, and evaluating efficacy and effectiveness. In brief, this approach proffers the sine qua non, the essential and minimum sufficient set of steps for building a logic-based process based on the best evidence that is adaptable adaptable and generalizable across the health care domains.

In brief, this approach, developed and advocated by AHRQ for dissemination of the best evidence, integrates and links the fields of basic science, evidence-based health care and comparative effectiveness research. In that context, it is important to note the principal threads of intervention against Dengue currently address the patients' socio-economic status (i.e., living conditions), community-based (i.e., educational) interventions, as well as biological (e.g., immunotherapies) and medical interventions. In brief and as in the case of VHFs, in experiencing Dengue local people employ multiple explanatory models to make sense of and respond to the syndemic nature of any Dengue outbreak. Local and indigenous epidemic control measures are often implemented and these are consistent with the ones being promoted by healthcare workers; although some cultural practices may amplify the outbreak. Improving treatment of VHFs in tropical regions prone to dengue ultimately hinges on effective and compassionate care for the effected patient. To this end, there is the need to refocus efforts on aggressive supportive care and clinical monitoring; including communications and social mobilization experts as a primary part of every outbreak response team; and reestablishing the isolation ward as the key functional component of the overall outbreak control strategy. Even in the context of vaccination programs, they must be tailored to regional and national epidemiological specificities. Introduction of Dengue vaccination in the national immunization programs must take into account the special features of each country without jeopardizing the existing vaccines already in use.

To be clear, vector control cannot be the only intervention to prevent or contain dengue. It is equally necessary to empower the communities to be better prepared to protect themselves against the mosquito infestation. Empowerment of the stakeholders may be obtained, for example, by means of raising the level of awareness and general knowledge. A study of the comparative effectiveness analysis has examined the annual targeted *larvae*-formation of standing water to neutralize the dengue vector *A. aegypti*. The 4-year campaign ended in 2005, and was centered in two urban areas of Cambodia with a population of 2.9 million people. Cost effectiveness, calculated as the ratio of disability adjusted life years (DALYs) saved to the net cost of the intervention, and interpreted following sensitivity analysis of the effectual range of study parameters, compared the intervention against the hypothetical alternative of no intervention. The results demonstrated that the simple step of larviciding standing water to neutralize the dengue vector reduced the number of dengue cases and deaths by 53%, decreased dengue hospitalizations annually by close to 3000, and dengue ambulatory cases by close to 12,000 cases. Overall, the intervention cost over $500,000, but resulted in a saving of 997 DALYs per year by averting medical care, which translated to an effective reduction in the cost of the intervention of about $200,000 yearly. More importantly, annual, targeted larviciding campaigns appear to be cost-effective medium-term interventions to reduce the epidemiologic and economic burden of dengue in urban areas of Cambodia **[25].**

Conclusion:

In conclusion, translational research yields an increasing understanding of the fundamental molecular immunobiology that results from infection with DENV, and unveils the modes by which dengue virus escapes immune surveillance processes. Translational effectiveness seeks to understand and to uncover the best available evidence for immune-based treatment interventions, and to integrate this evidence in evidence-based decisions within specific clinical settings on site. Translational effectiveness for medical interventions to contain and control the pathologies that result from DENV infection relies on the consensus of the evidence produced by systematic reviews. Moreover, considering the several candidate Dengue vaccines under development, it is timely and critical to assist stakeholders to better understand the potential economic value and cost effectiveness of Dengue vaccines, one provisional goal is that vaccination may replace environmental control as a strategy for cost and life- saving dengue prevention modality. As we go forward in the next few years, the joint consideration of translational research and translational effectiveness concerns in the context of the novel model of translational science in health care in general, and patient-target Dengue intervention in particular will benefit patients, caregivers and stakeholders along the complex syndemic dimensions of this viral disease.

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